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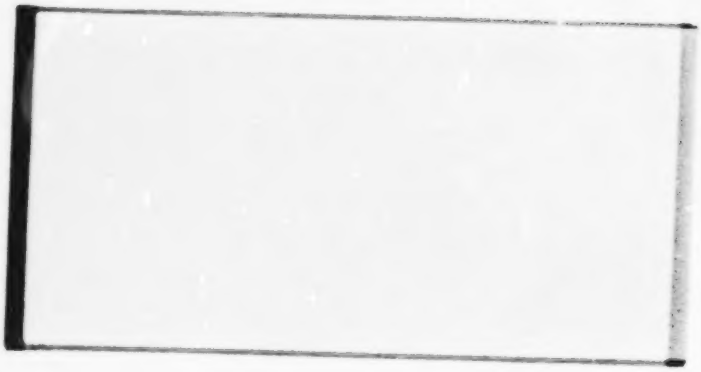

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EFFECTS OF THE HERBICIDES 2,4-D PLUS 2,4-DP; 2,4-D PLUS PICLORAM; AND TRICLOPYR ON ASH AND POPLAR

Report No 83-88-K

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ABSTRACT

The effects of foliar-application of the three herbicide treatments 2,4-D plus 2,4-DP, picloram plus 2,4-D, and triclopyr on leaf and young stem appearance, stomatal diffusion resistance, apparent photosynthesis, leaf respiration and root respiration were determined on Carolina poplar and white ash trees grown in the greenhouse. Carolina poplar trees were sensitive to all three herbicide treatments in that foliar and young stem death had occurred within 15 days of herbicide application. After 45 days, the roots of Carolina poplar were still alive, although root respiration was partially inhibited by all three herbicide treatments indicating translocation of herbicide from the leaves to the roots but in insufficient quantities to kill the latter. The white ash trees were more herbicide-resistant than Carolina poplar, in that the mixture of 2,4-D plus 2,4-DP had little or no effect on stomatal diffusion resistance, apparent photosynthesis and root respiration. Of these three metabolic processes, picloram plus 2,4-D inhibited the latter. However, triclopyr inhibited both apparent photosynthesis and root respiration. By 17 days after application, all three herbicide treatments had induced foliar and young stem death. However, even after 45 days, the roots of white ash were still alive (triclopyr and picloram plus 2,4-D had caused a partial inhibition of root respiration).

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EXECUTIVE SUMMARY

EFFECTS OF THE HERBICIDES 2,4-D PLUS 2,4-DP; 2,4-D PLUS PICLORAM; AND TRICLOPYR ON ASH AND POPLAR

G.P. Arron
Biological Research Section
Chemical Research Department

Ontario Hydro employs the herbicides 2,4-D (2,4-dichlorophenoxy-acetic acid), 2,4-DP (2,4-dichlorophenoxypropionic acid), picloram (4-amino-3,5,6-trichloropicolinic acid) and sodium TCA (sodium trichloroacetate) to control brush and woody species on rights-of-way. Combinations of these herbicides are applied as foliar, basal or stump treatments to woody plant material.

Certain woody species, such as ash, are more resistant to these herbicides than are others (eg poplar). In this report the effects of foliar-application of the herbicides 2,4-D plus 2,4-DP and picloram plus 2,4-D, and triclopyr (3,5,6-trichloro-2-pyridinyloxy acetic acid) (a new herbicide, not used by Ontario Hydro at present) on various aspects of the metabolism and young stem and foliar appearance of a herbicide-resistant tree species (white ash) and a herbicide-sensitive tree species (Carolina poplar) are described. Carolina poplar trees were sensitive to all three herbicide treatments, causing an increase in stomatal diffusion resistance and an inhibition of apparent photosynthesis and root respiration. Within 15 days of herbicide application, foliar and young stem death had occurred. After 45 days the roots of Carolina poplar were still alive, although root respiration was partially inhibited (by all three herbicide treatments) indicative of translocation of herbicide or phytotoxic herbicide metabolite from the leaves to the roots. The white ash trees were more herbicide-resistant than Carolina poplar, in that the mixture of 2,4-D plus 2,4-DP had little or no effect on stomatal diffusion resistance, apparent photosynthesis and root respiration. Of these three metabolic processes, picloram plus 2,4-D inhibited the latter. However, triclopyr inhibited both apparent photo-synthesis and root respiration. By 17 days after application,

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all three herbicide treatments had caused foliar and young stem death (defoliation did not occur until later). After 45 days, the roots of white ash appeared largely unaffected (both triclopyr and picloram plus 2,4-D had caused a partial inhibition of root respiration).

Although the mixtures of 2,4-D plus 2,4-DP and picloram plus 2,4-D, and triclopyr were effective in inducing the rapid death of the foliage and young stems of both Carolina poplar and white ash, all the herbicides were ineffective in killing the roots of either species (at least by 45 days after application). The long-term effects of these herbicides on the roots of Carolina poplar and white ash, and their relationship to the efficiency of translocation of the herbicide from the leaves to the roots remain to be investigated. The primary objective in woody plant control is the destruction of the complete root system, since inefficient root kill will result in stem sprouting and root suckering following treatment.

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To Mr. F.J. Kee
Director of Research

EFFECTS OF THE HERBICIDES 2,4-D PLUS 2,4-DP; 2,4-D PLUS PICLORAM; AND TRICLOPYR ON ASH AND POPLAR

1.0 INTRODUCTION

Ontario Hydro uses various herbicides to control brush and woody species on rights-of-way. The most commonly used herbicides are 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DP (2,4-dichlorophenoxypropionic acid) and picloram (4-amino-3,5,6-trichloropicolinic acid). Combinations of these herbicides (eg 2,4-D plus 2,4-DP and picloram plus 2,4-D) are applied as foliar, basal or stump treatments to the plant material. Certain woody species encountered on rights-of-way, such as white ash, are more resistant to these herbicides than are others (eg poplar).

The different responses of woody species to the same herbicide can usually be explained in terms of morphological or physiological differences. The phenoxy herbicides (such as 2,4-D and 2,4-DP) have a complex, probably multisite mechanism of action and their application to plants results in a wide variety of responses (for a description see Ontario Hydro Research Division Report No 82-153-K). Determination of the effects of foliar-application of those herbicides used by Ontario Hydro on various fundamental physiological processes (eg respiration, photosynthesis) that occur in the leaves and/or the roots of a herbicide-resistant woody species will aid in the discovery of the basis of that resistance.

In the experiments described in this study, the effect of the herbicide (mixtures) 2,4-D plus 2,4-DP, picloram plus 2,4-D and triclopyr (a new herbicide produced by Dow Chemical Company, not used by Ontario Hydro) on various aspects of the metabolism and on the external appearance of young stems and leaves of a herbicide-resistant tree species (white ash) and a herbicide-sensitive tree species (Carolina poplar) was determined. The various herbicides were applied to the tree material as a foliar-spray, in a manner as similar to that used in the field situation as possible (ie, similar herbicide concentration, volume of spray applied and method of application). At various intervals after herbicide application, the appearance of the trees was noted, the rates of photosynthesis, leaf respiration and root respiration determined, the stomatal diffusion resistance measured and samples of tree tissue were retained for examination by light microscopy.

2.0 MATERIALS AND METHODS

2.1 Plant Material

Two-year old white ash (*Fraxinus americana* L.) and Carolina poplar (*Populus canadensis* Moench) trees were a gift from the Ontario Ministry of Natural Resources (collected from the Midhurst station). The former were about 0.5 m in height and the latter, about 2 m. The white ash trees were planted in 30 cm pots in a one to one mixture of shredded topsoil (obtained locally) and peat moss (Plant Products, Bramalea, Ontario), and the Carolina poplar trees were planted in 37 cm pots in the same mixture. The trees were placed in the greenhouse immediately after potting and watered at regular intervals. At weekly intervals the topsoil in each pot was dug over and a fertilizer applied (solution of 20-20-20 (NPK) obtained from Plant Products). Approximately one to two weeks after planting 95% of both the white ash and Carolina poplar trees had broken dormancy. Herbicides were applied to the trees five to six weeks after dormancy breakage. Forty eight trees of each species were used for each herbicide treatment, and forty eight for the control.

2.2 Herbicide Application

The herbicides picloram plus 2,4-D and triclopyr were obtained from Dow Chemical Company, Sarnia, Ontario; and the mixture of 2,4-D and 2,4-DP was obtained from Union Carbide, Chatham, Ontario.

For a detailed description (full chemical name, concentration) of the herbicides used, see Appendix A.

The stock mixture of 2,4-D and 2,4-DP, and the stock mixture of picloram and 2,4-D were both diluted 100 times with tap water to give a 1% solution, before application to the trees. Triclopyr was diluted 133 times to give a 0.75% solution. (In vegetation control on rights-of-way, Ontario Hydro currently applies a 1% solution of both 2,4-D plus 2,4-DP and picloram plus 2,4-D to tree and brush foliage. The stock mixtures of herbicides are of the same concentration as used in the experiments described here. Triclopyr is not used by Ontario Hydro on rights-of-way at present.)

The herbicides were applied to the tree foliage (to leaf drip) using a small hand sprayer at 25 psi with a Tee Jet 8004 nozzle. The pots were covered with plastic sheets to prevent excess herbicide dripping onto the soil surface. After spraying, the trees were returned to one compartment of the greenhouse; unsprayed (control) trees were located in the other compartment.

2.3 Observations on the Effect of the Herbicides Used on the Appearance of Carolina Poplar and White Ash

At regular intervals, notes were made on the appearance of the treated trees. Discolouration of leaves or stems, and loss of turgor in leaves or stems were recorded using a 4 stage scoring system (0 - no effect, 3 - maximum effect). A score of 3 for leaf discolouration, meant that approximately 95% of the surface of 95% of the leaves were discoloured. The subjective element of such a scoring system should be taken into account during interpretation of the results. Contact

damage (burn marks caused by droplets of herbicide solution on the leaf surface) were also assessed.

2.4 Determination of Stomatal Diffusion Resistance

Stomatal diffusion resistance was measured on the upper and lower surfaces of the same leaf using an autoporometer (LI-65 Lambda Instruments Corporation, Lincoln, Nebraska). At each time interval, the stomatal diffusion resistance was measured in three leaves chosen at random. The autoporometer was calibrated as described in the equipment manual (Lambda Instruments A-176) and the calibration curve used to correct the experimental data to 25°C. Towards the end of the experiment, the poor physical condition of the experimental leaves (dry and fragile) meant that some measurements could not be taken. Defoliation did not occur until some time after the leaves became dry and fragile.

2.5 Determination of Leaf Photosynthesis

In simple terms photosynthesis consists of the reduction of atmospheric CO_2 to carbohydrate by use of light energy, with an associated release of oxygen from water. The reaction can be summarized by the following generalized equation:

$$CO_2 + 2H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} (CH_2O) + O_2 + H_2O.$$

The oxygen produced is released to the atmosphere. Oxygen evolution from discs cut from Carolina poplar and white ash leaves was monitored at 30°C using a Rank oxygen electrode system (Rank Bros, Bottisham, Cambridge, UK). Three leaf discs, cut with a No 5 cork borer (CanLab), from three leaves chosen at random from three trees, were placed in 2 mL of 100 mM potassium phosphate buffer (pH 7.0) and 10 mM $NaHCO_3$ (a source of CO_2 for the above reaction). The potassium phosphate buffer had been bubbled with dry nitrogen for several minutes to remove the oxygen. The oxygen electrode was calibrated using a sample of potassium phosphate buffer equilibrated with air at 30°C (1 mL of air-saturated medium was taken to contain 235 nmoles of O_2 at 30°C - calculated from Umbreit et al, 1964). Illumination of the oxygen electrode was provided by two 150-W spot lamps providing a total quantum flux density of approximately 8000 $\mu E\ m^{-2}\ s^{-1}$ (between 400 and 700 nm) at the outside of the electrode water-jacket (measured with Lambda Instruments Model LI-185A light meter). Large flat flasks filled with water were placed between the spot lamps and the oxygen electrode to act as heat shields. Changes in oxygen tension were recorded on a single-pen chart recorder (Linear Instruments). Results were expressed as nmoles O_2 evolved per minute per square cm of leaf. Towards the end of the experiment, some of the herbicide treated material did not evolve oxygen in the photosynthesis assay, but consumed oxygen (ie respiration was taking place - see below). In this case, the results are expressed as a negative rate of oxygen evolution. In the photosynthesis assay described above, net photosynthesis is measured (as opposed to gross

photosynthesis). Respiration occurs in leaves in the light and dark, and so some oxygen is being consumed at all times. Net (or apparent) photosynthesis may be summarized thus:

Net (or apparent) photosynthesis

= Gross photosynthesis - respiration.

Thus, when the rate of gross photosynthesis is low, and the respiration rate is higher, the net effect is an uptake of oxygen. At the compensation point, the rate of gross photosynthesis is equal to the rate of respiration and thus net photosynthesis is zero.

It should also be stressed that the rates of photosynthesis measured in the above assay, are determined under standardized conditions (light, temperature, CO_2 concentration), and if photosynthesis were measured in intact leaves still on the tree, these conditions would vary from day to day with concomitant changes in the rates of photosynthesis measured. In this latter situation, it would be difficult to differentiate between environmental effects on the rate of photosynthesis and herbicide effects. A similar situation will exist when leaf respiration is measured (see below), although environmental effects on respiration will be less pronounced (for a detailed discussion of the factors affecting photosynthesis and respiration, see Kramer and Kozlowski (1979)).

2.6 Determination of Leaf Respiration

Oxygen consumption by leaf discs was measured at 30°C in a Rank oxygen electrode system. Three leaf discs cut from three leaves chosen at random were placed in 2 mL of 100 mM potassium phosphate buffer (pH 7.0). The phosphate buffer had been air-equilibrated at 30°C by placing it in the circulating-water system used to control the temperature of the oxygen electrode. Rates of leaf respiration are expressed as nmoles O_2 consumed per minute per square cm of leaf.

2.7 Determination of Root Respiration

Oxygen consumption by root segments was measured at 30°C in the same way as leaf respiration was determined (see above). Samples of washed tree roots were cut into short sections and placed in 2 mL of 100 mM potassium phosphate buffer (pH 7.0). After assay the root segments were removed, surface dried and weighed. Oxygen consumption was expressed as nmoles O_2 taken up per minute per g fresh weight.

2.8 Light Microscopy

Samples of two plants for each species and treatment were taken at intervals of 5 and 13 days after herbicide application. Portions of petioles of upper leaves were fixed in FAA (formalin - acetic acid - alcohol), dehydrated with a graded t-butyl alcohol series, embedded in Tissue Mat and sectioned with a Clinical AO microtome. Slides were stained with a mixture of safranin and fast green.

3.0 RESULTS AND DISCUSSION

3.1 Effects of the Herbicides 2,4-D plus 2,4-DP; Picloram plus 2,4-D; and Triclopyr on the Appearance and Xylem Vessel Fine Structure of White Ash and Carolina Poplar Trees

The effects of the herbicides (mixtures) 2,4-D plus 2,4-DP; picloram plus 2,4-D; and triclopyr on the appearance of Carolina poplar and white ash trees are summarized in Tables 1 and 2 respectively. In general the effects of all the herbicides on Carolina poplar were similar (see Table 1) although the time scale for the appearance of discoloured leaves and stems, and loss of turgor in leaves and stems varied. The mixture of 2,4-D plus 2,4-DP caused the most rapid development of the above effects, followed by triclopyr and then the picloram plus 2,4-D mixture. After 11 to 15 days, all the experimental leaf material was dead, although defoliation did not occur until later.

Appearance of foliar and stem damage after spraying of white ash trees with the various herbicides was slower than with the Carolina poplar (Table 2). Again 2,4-D plus 2,4-DP appeared to cause the most rapid development of foliar and stem injury, followed by triclopyr and then picloram plus 2,4-D. With the exception of picloram plus 2,4-D, herbicide-induced foliar and stem damage appeared maximal between 17 and 29 days after herbicide application and at 29 days some defoliation was apparent.

Various investigators have described the rapid development of a permanently wilted condition in plants after the application of 2,4-D (see Bradbury and Ennis, 1952). In their review, Robertson and Kirkwood (1970) quote the characteristic symptoms of phenoxy-herbicide (eg 2,4-D) damage as a proliferation of tissue resulting in leaf epinasty, swelling and epinasty of stems. In leaf and stem epinasty, uneven growth leads to a downward bending of the leaf and stem and this resembles a loss of turgor. The downward bending of leaves and stems observed in the Carolina poplar and white ash trees (Tables 1 and 2), which was described as loss of turgor, may have been leaf and stem epinasty, although in some cases the response was observed only 2 days after herbicide application, and such a rapid response might not be growth related.

The distortions of growing stems and leaves in picloram-treated plants are reported to be similar to those produced by 2,4-D (Fisher et al, 1968). Both picloram and 2,4-D have been shown to reduce the chlorophyll content of herbicide-sensitive plants (Sharma and Vanden Born, 1972; Bruinsma, 1961) and this would result in discolouration, as observed in Carolina poplar and white ash (Tables 1 and 2).

Examination by light microscopy of a series of transverse sections of leaf petioles taken from Carolina poplar and white ash trees that had received a foliar-application of 2,4-D plus 2,4-DP;

TABLE 1

OBSERVATIONS ON THE EFFECT OF FOLIAR-APPLICATION OF THE HERBICIDES 2,4-D PLUS 2,4-DP; PICLORAM PLUS 2,4-D AND TRICLOPYR ON THE APPEARANCE OF YOUNG CAROLINA POPLAR TREES

The various trees were scored on a scale of 0 (no effect) to 3 (maximum effect) at the times indicated.

Herbicide Applied	2,4-D + 2,4-DP					Picloram+2,4-D					Triclopyr				
Days after Herbicide Applied	2	7	11	17	29	2	7	11	17	29	2	7	11	17	29
Effects Observed:															
Discolouration - young leaves†	2	3	3	3	3	0	1	2	3	3	2	2	2	3	3
- old leaves	2	3	3	3	3	0	1	2	3	3	2	2	2	3	3
Discolouration - young stem†	0	0	1	3	3	0	1	2	3	3	0	1	3	3	3
Loss of turgor - leaves	2	3	3	3	3	2	3	3	3	3	2	2	3	3	3
- young stem†	2	3	3	3	3	0	0	2	3	3	0	0	1	3	3
Contact damage to leaves*	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
Defoliation	0	0	0	0	2	0	0	0	0	2	0	0	0	0	2
Comments	Leaves became discoloured except in area next to veins. All leaves dead at 15 days.					All leaves dead at 15 days					All leaves dead at 15 days				

* Contact damage describes the burn marks caused by droplets of herbicide which landed on the leaves. Not all leaves exhibited such burn marks, because not all leaves received herbicide (some leaves were shielded by others during the spraying operation). In the case of poplar, discolouration eventually affected all leaves, regardless of whether each leaf had contact damage on it. Presumably this reflects an efficient transfer of herbicide from one leaf to another.

† Young stems and leaves refers to plant material in existence before herbicide application.

TABLE 2

OBSERVATIONS ON THE EFFECT OF FOLIAR-APPLICATION OF THE HERBICIDES 2,4-D PLUS 2,4-DP: PICLORAM PLUS 2,4-D AND TRICLOPYR ON THE APPEARANCE OF YOUNG WHITE ASH TREES

The various trees were scored on a scale of 0 (no effect) to 3 (maximum effect) at the times indicated.

Herbicide Applied	2,4-D + 2,4-DP					Picloram + 2,4-D					Triclopyr				
Days after Herbicide Applied	2	7	11	17	29	2	7	11	17	29	2	7	11	17	29
Effects Observed:															
Discolouration - young leaves†	1	2	3	3	3	0	1	2	2	2	1	1	2	3	3
- old leaves	0	1	3	3	3	0	1	2	2	2	0	0	1	3	3
Discolouration - young stems†	0	1	1	2	3	0	0	0	1	1	0	0	1	3	3
Loss of turgor - leaves	1	1	1	3	3	1	1	2	2	2	1	1	2	3	3
- young stem†	0	0	1	3	3	0	1	2	2	2	0	0	1	2	3
Contact damage to leaves*	2	2	2	2	2	1	1	1	1	1	2	2	2	2	2
Defoliation	0	0	0	0	2	0	0	0	0	2	0	0	0	0	2
Comments	Young leaves became discoloured and lost turgor before older leaves. At 17 days most leaves dead.					At 29 days some leaves remained on the trees, and of these a few were turgid and green with some discolouration.					At 17 days most leaves were dead. At 29 days some leaves remained on the trees, but were brown and dry; the young stems were severely damaged.				

* Contact damage (described in the note to Table 1) was observed in ash saplings sprayed with triclopyr, at least 7 days before leaf discolouration became widespread in young and old leaves (cf effect of triclopyr on poplar leaves).

† Young stems and leaves refers to plant material in existence before herbicide application.

picloram plus 2,4-D; and triclopyr either 5 or 13 days previously, revealed that neither herbicide treatment appeared to have any effect on the xylem vessels. Previous work by Peterson et al (1974) has shown that presentation of picloram to the roots resulted in xylem element blockage in red maple (a herbicide-sensitive species) whilst white ash (a herbicide-resistant species) showed no such blockages. These authors suggested that xylem element blockage led to impeded upward movement of water and leaf desiccation, which ultimately led to foliar death (Peterson et al, 1974). The effect picloram has on xylem elements may vary depending on the place of entry of the herbicide into the plant, ie root or leaf.

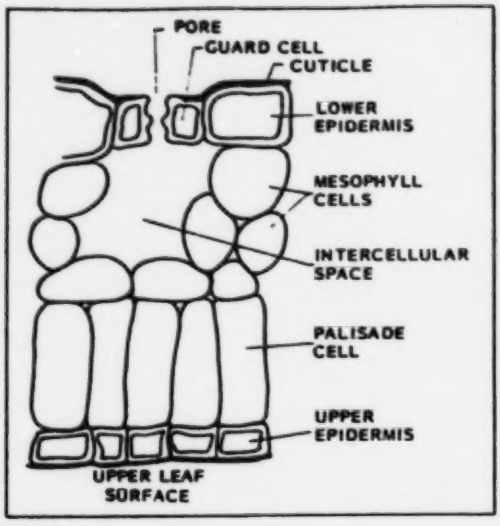
3.2 Effects of the Herbicides 2,4-D plus 2,4-DP; Picloram plus 2,4-D; and Triclopyr on the Stomatal Diffusion Resistance of Leaves of Carolina Poplar and White Ash Trees

The relationship between stomatal diffusion resistance and transpiration in photosynthetic tissue requires a brief explanation. Carbon dioxide, one of the substrates for photosynthesis, enters the leaf mainly via the stomata (or pores) on the leaf surface, and then dissolves in the water covering the mesophyll cells (Kramer and Kozlowski, 1979). As carbon dioxide enters the leaf, water vapour leaks out through the stomata and cuticle and this is termed transpiration. Transpiration may be beneficial to the plant because it cools leaves, causes the ascent of sap, and increases absorption and translocation of minerals (Kramer and Kozlowski, 1979).

Seybold (1929, 1930) was the first to introduce the reciprocal of transpiration rate as a transpiration resistance, measured under standard conditions of evaporation. The diffusional portion of the water vapour transfer in transpiration, ie the transfer of water vapour by molecular diffusion in still air, is localized partly in the plant itself (intercellular spaces, stomata) and partly in the boundary layer of air at the surface of the plant:

$$r_{total} = r_{\ell} + r_a$$

where r_{total} is the sum of the transpiration resistances, r_{ℓ} is the resistance situated in the leaf and r_a is the resistance of the boundary layer. The leaf resistance r_{ℓ} consists of two serial diffusive resistances involved in stomatal transpiration: the intercellular resistance (r_i) in still intercellular air and the stomatal pore resistance (r_{pore}), and a parallel resistance involved in the cuticular component of transpiration: the cuticular resistance (r_c). (Another resistance, r_m, the mesophyll cell wall resistance is thought to be very low. Farquhar and Raschke (1978) found no mesophyll cell wall resistance in several herbaceous species and concluded that the water vapour pressure at the evaporating surfaces is equal to its saturation vapour pressure.) A diagram of these various resistances in a leaf and their relationship is shown in Figure 1. In moving air the boundary layer resistance r_a



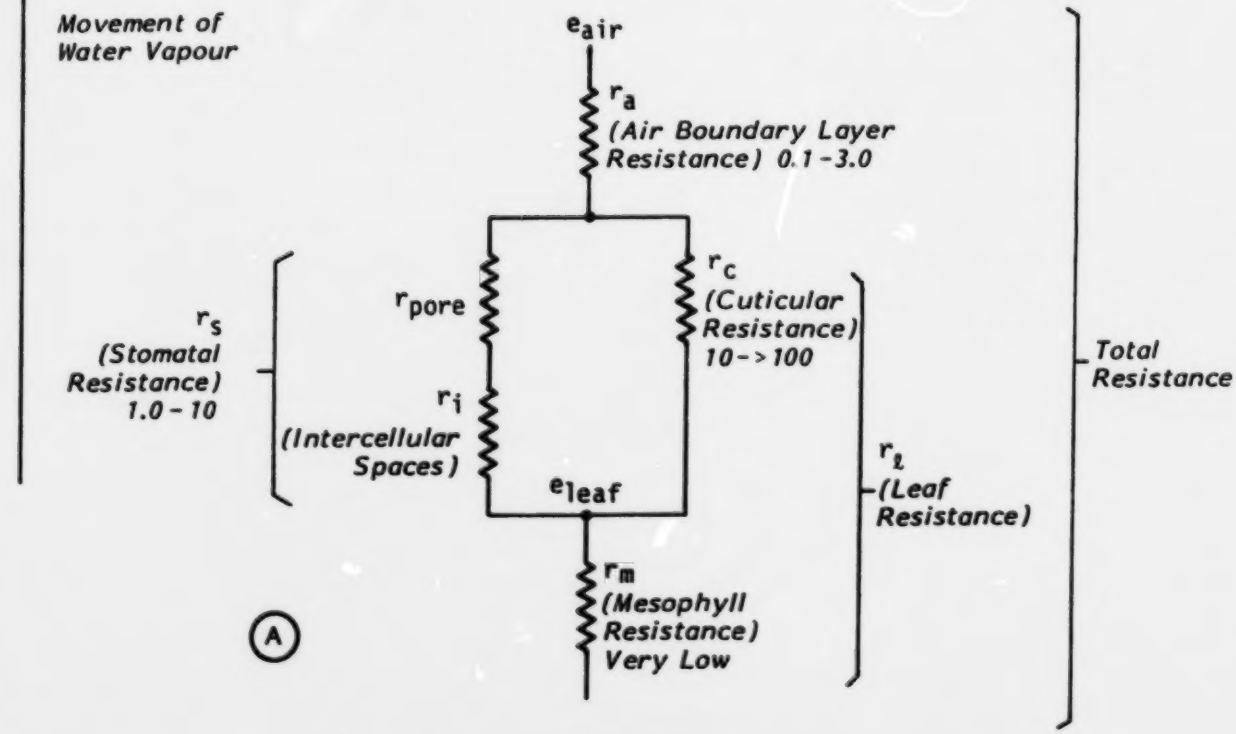


FIGURE 1

DIAGRAM SHOWING RESISTANCES TO THE DIFFUSION OF WATER VAPOUR FROM A LEAF

A: *Schematic Diagram* **B**: *Cross-Section of Leaf*
The rate of transpiration is proportional to the steepness of the gradient in water vapour pressure, e_{leaf} to e_{air}, and is inversely proportional to the resistances measured in seconds per centimeter. In moving air, r_a will be small and there will be a strong (inverse) relationship between stomatal resistance and transpiration.
(Figure taken from Kramer and Kozlowski, 1979)

is extremely low, and the stomatal resistance r_s may be taken as a measure of the transpiration rate (one is inversely proprotional to the other).

The effects of the various foliar-applied herbicides on the stomatal diffusion resistance of Carolina poplar leaves are shown in Table 3. The values given are for the whole leaf (the upper leaf surface plus the lower). The stomatal diffusion resistance values for the lower leaf surface were generally smaller than those obtained for the upper leaf surface (see Appendix B), and this is related to the larger number of stomata on the lower surface (Kramer and Kozlowski, 1979). Over the course of the experiment, the control values for the whole leaf stomatal diffusion resistance (r_s) were low, ranging from 2.5 ± 0.9 to 6.5 ± 1.6 sec cm^{-1} (Table 3). One day after application of either of the herbicides (mixtures), the stomatal diffusion resistance values obtained were generally higher than those found for untreated material. This suggested that transpiration had been inhibited by all three herbicide combinations. By 15 days, all the leaves on the herbicide-treated Carolina poplar trees were dead.

It should be noted that high values for whole leaf stomatal diffusion resistance are inaccurate, in that when water vapour is escaping from the leaf very slowly, any leaks in the measuring apparatus will introduce large errors into the observed values. When water vapour escapes from the leaf quickly (ie the stomata are open), then such leaks become insignificant. Typical values for whole leaf stomatal diffusion resistance when the stomata are closed, and water vapour is escaping through the cuticle, range from 20 to 40 sec cm^{-1}, and vary from species to species. The value for $r_{s(total)}$ for Carolina poplar when the stomata are closed, seems to be in this range, since 7 days after application of 2,4-D plus 2,4-DP a mean value of 20.2 ± 5.4 sec cm^{-1} was recorded (Table 3). The leaves were in poor physical condition, being discoloured and lacking turgor (see Table 1) and apparent photosynthesis was inhibited (see below). Since values for $r_{s(total)}$ above approximately 30 sec cm^{-1} are inaccurate, this may be reflected by the large standard deviation from the mean found in some treatments which have a high whole leaf stomatal diffusion resistance (and thus a low rate of transpiration).

The effects of the various herbicide treatments on the stomatal diffusion resistance of leaves of white ash were not so pronounced. The control values for whole leaf stomatal diffusion resistance in white ash were higher than those obtained with Carolina poplar (compare Tables 3 and 4), with a mean value over the course of the experiment of 11.3 sec cm^{-1}. (For some reason the whole leaf stomatal diffusion resistance measured in control white ash trees was high after 11 days and low after 15 days (21.1 ± 7.0 and 2.2 ± 0.5 sec cm^{-1} respectively). Although this cannot be explained, there was some consistency in the data in that the experimental values were also high on day 11 and low on day 15.) The upper leaf surface stomatal diffusion resistance of white ash control trees

TABLE 3

EFFECT OF THE FOLIAR-APPLIED HERBICIDES 2,4-D PLUS 2,4-DP; PICLORAM PLUS 2,4-D; AND TRICLOPYR ON THE STOMATAL DIFFUSION RESISTANCE (r_s) OF LEAVES OF CAROLINA POPLAR TREES

Stomatal diffusion resistance of the upper and lower surface of the same leaf was measured on three leaves chosen at random as described in Materials and Methods. Total (whole leaf) stomatal diffusion resistance was calculated using the formula (from Slavik, 1974)

$$\frac{1}{r_{s(total)}} = \frac{1}{r_{s(upper)}} + \frac{1}{r_{s(lower)}}$$

Data given below are the mean ± one standard deviation. All the data collected are tabulated in Appendix B. At 15 days all the experimental leaves were dead.

	Days after Herbicide Applied				
Herbicide Applied	1	2	4	7	9
	$r_{s(total)}$ (sec cm^{-1})				
Control	5.6±1.1	4.1±0.9	6.5± 1.6	2.6± 0.5	2.5± 0.9
2,4-D + 2,4-DP	40.0±4.5	23.3±11.2	37.6±17.3	20.2± 5.4	70.5± 7.3
Picloram + 2,4-D	37.6±9.3	21.0±15.9	12.9± 7.3	39.9± 1.3	216.7± 9.9
Triclopyr	26.1±4.3	26.5± 8.5	38.5±15.3	30.1±10.1	33.3±11.9

TABLE 4

EFFECT OF FOLIAR-APPLIED HERBICIDES 2,4-D PLUS 2,4-DP; PICLORAM PLUS 2,4-D; AND TRICLOPYR ON THE STOMATAL DIFFUSION RESISTANCE (r_s) OF LEAVES OF WHITE ASH TREES

The conditions were the same as described in the legend to Table 3. Data given below is the mean ± one standard deviation. All the data collected are tabulated in Appendix C. Where no values are given the leaves were in too poor physical condition to allow measurement.

Herbicide Applied	Days after Herbicide Application								
	1	2	4	7	9	11	15	25	30
	$r_{s(total)}$ (sec cm^{-1})								
Control	13.4±5.5	11.3± 9.2	8.2± 3.7	9.3± 5.9	15.7± 6.6	21.1± 7.0	2.2± 0.5	7.6± 2.8	12.7± 5.5
2,4-D + 2,4-DP	22.1±8.4	34.4± 4.5	22.9± 1.8	32.2± 8.4	37.0±17.2	53.6±27.3	26.8±10.2	26.4± 9.9	-
Picloram + 2,4-D	8.9±1.0	15.7±10.4	29.4±10.2	28.9± 5.7	24.0± 8.7	27.2± 9.2	11.9± 5.5	12.6± 4.0	28.7± 5.4
Triclopyr	11.2±5.2	36.3±20.2	24.3±13.5	49.4±15.3	37.7±21.9	62.0±18.7	-	-	-

was extremely high (compared to lower leaf surface - see Appendix C) and this reflects the paucity of stomata on the upper surface (Kramer and Kozlowski, 1979).

Application of all the herbicide treatments to white ash appeared to cause an increase in the whole leaf stomatal diffusion resistance, but the magnitude of the increase was small compared to that observed with Carolina poplar, and the data obtained were sometimes inconsistent (Table 4). Initially both 2,4-D plus 2,4-DP and picloram plus 2,4-D had little or no effect, and not until after two and four days respectively, was a modest increase seen. Triclopyr also appeared to cause a modest increase in the stomatal diffusion resistance, and this was not seen until the second day.

Whilst all the herbicides examined had a rapid inhibitory effect on the rate of transpiration of Carolina poplar trees (a herbicide-sensistive tree species), their effect on the rate of transpiration of white ash trees (a herbicide-resistant tree species) was not so pronounced, with a far less obvious inhibition which took longer to manifest itself. These results agree with the early work of Kasperlik (1955), who found that 2,4-D caused greatly reduced rates of transpiration (due to effects on the stomatal mechanism) in herbicide sensitive plants such as broad bean (*Vicia faba* L.) and white mustard (*Sinapis alba* L.), where there was no change in the rate with herbicide-resistant plants such as oats (*Avena sativa* L.). Bradbury and Ennis (1952) had earlier reported that foliar application of ammonium 2,4-D caused partial stomatal closure in red kidney bean, and this was interpreted as a direct effect of the herbicide on the leaves rather than an indirect effect on the water-absorbing mechanism of the roots. An inhibition of transpiration (ie the upward movement and subsequent loss of water) appears to lead to the inhibition of the downward movement of photosynthetic products from the leaves to the roots via the phloem, and thus the translocation of foliar-applied herbicides or phytotoxic herbicide metabolite to the roots may be inhibited also (Cronshaw, 1981).

3.3 Effects of the Herbicides 2,4-D plus 2,4-DP; Picloram plus 2,4-D; and Triclopyr on the Apparent Photosynthesis of Carolina Poplar and White Ash Trees

The effect of the various herbicides on the rate of apparent photosynthesis of leaf discs from Carolina poplar trees is shown in Figure 2. The mixture of 2,4-D and 2,4-DP caused an inhibition of apparent photosynthesis, evident one day after foliar-application. Both picloram plus 2,4-D and triclopyr had no such immediate effect after foliar-application, but after 7 days apparent photosynthesis was totally inhibited by both treatments. By 15 days after herbicide application (in the case of picloram plus 2,4-D, 9 days) the leaves on all the experimental trees were dehydrated and fragile, and measurements could not be made.

The rates of apparent photosynthesis in leaf discs from untreated white ash trees were generally lower than those observed in leaf discs from untreated Carolina poplar trees (compare control values Figures 2 and 3) and this is related to the faster rate of growth of the latter species (Kramer and Kozlowski, 1979). The control values for apparent photosynthesis in leaf discs from Carolina poplar and white ash trees decreased with time (see Figures 2 and 3), and this may be explained by the observation of Schaedle (1975) that rates of apparent photosynthesis increase during the expansion of the young tree leaf, and reach a steady-state condition of photosynthetic performance for 10 to 40 days depending on environmental conditions. With increasing leaf age apparent photosynthetic rates in trees decline (Schaedle, 1975).

Application of either 2,4-D plus 2,4-DP or picloram plus 2,4-D to white ash trees had little or no effect on the rate of apparent photosynthesis in isolated leaf discs (Figure 3). Triclopyr caused a rapid inhibition of apparent photosynthesis which was sustained for approximately 23 days, after which the leaves of the treated trees were dry and fragile. At each time interval, the values for the rate of apparent photosynthesis in triclopyr treated material were lower than the control values.

Ashton et al (1977) have reported that 2,4-D inhibited photosynthesis in red kidney beans (*Phaseolus vulgaris* L.), whilst carbon dioxide absorption by *Pinus resinosa* Ait seedlings was reduced by 2,4-D application (Sasaki and Kozlowski, 1967). Carbon dioxide absorption by both red maple (*Acer rubrum* L.) and white ash (*Fraxinus americana* L.) was inhibited by 2,4,5-T (Leonard et al, 1966). Penner and Ashton (1966) have described how 2,4-D stimulates, inhibits, or has no effect on both photosynthesis and respiration. The fact that the phenoxy-herbicides (eg 2,4-D) stimulate growth processes at low concentrations but inhibit them at high concentrations may be an explanation for this observation (for a discussion of this effect see Ontario Hydro Research Division Report 82-153-K).

Picloram has also been reported to inhibit the fixation of carbon dioxide by excised leaves of soybean (*Glycine max* L.), although Sharma and Vanden Born (1971) concluded that this was an indirect effect and was a result of disturbances of other physiological processes. Such an explanation has also been suggested to explain the effects of phenoxy-herbicides (eg 2,4-D) on both photosynthesis and respiration (Robertson and Kirkwood, 1970).

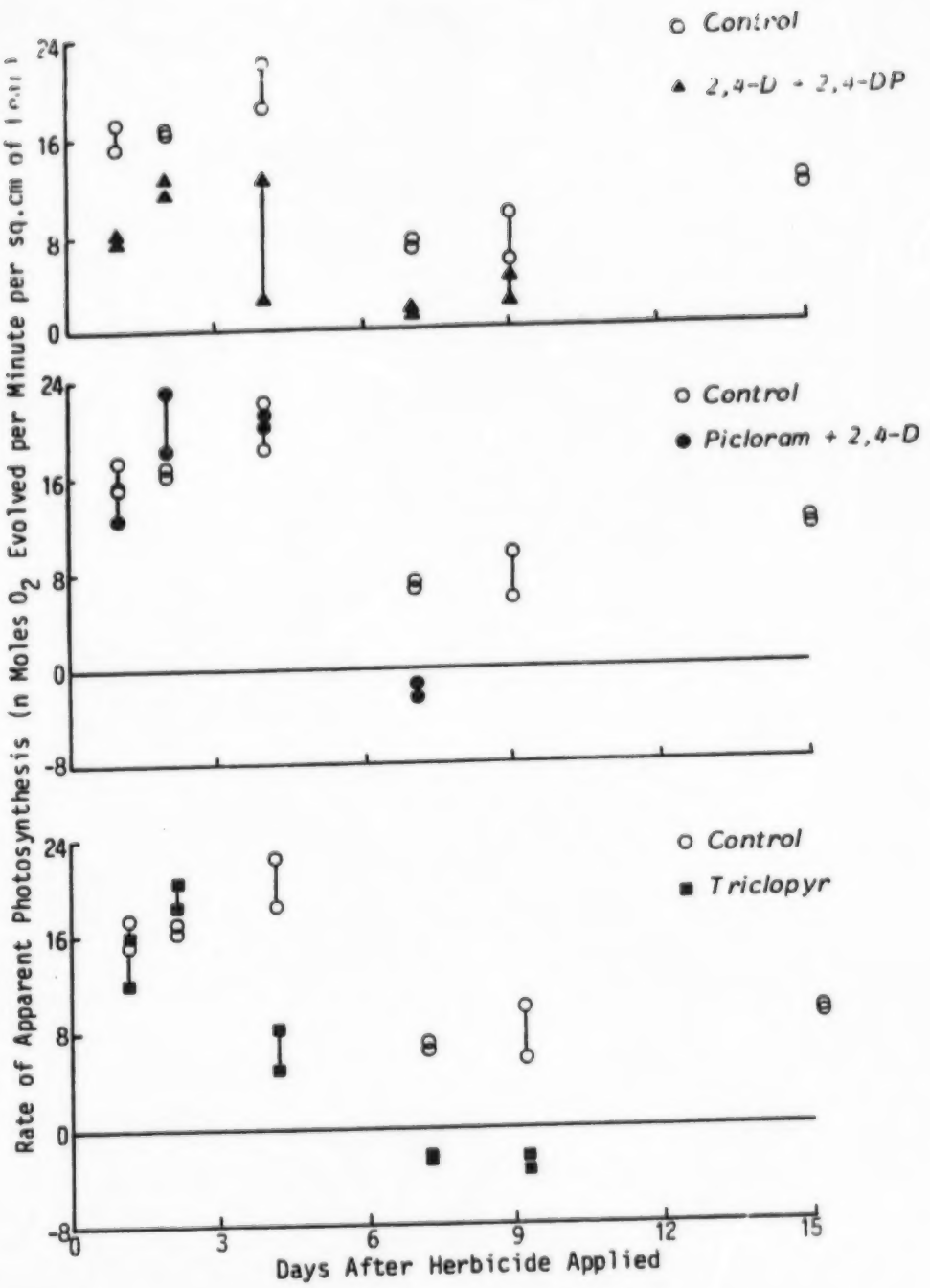


FIGURE 2

EFFECT OF VARIOUS HERBICIDES ON THE APPARENT PHOTOSYNTHESIS OF LEAF DISCS FROM CAROLINA POPLAR TREES

Photosynthesis was measured as described in Materials and Methods. A negative rate of oxygen evolution means that oxygen was consumed (i.e. respiration). Two measurements were made for each treatment at various time intervals, and these two values are plotted. At 15 days (and in the case of Picloram and 2,4-D at 9 days) no measurements could be made of treated materials because of the fragile condition of the leaves.

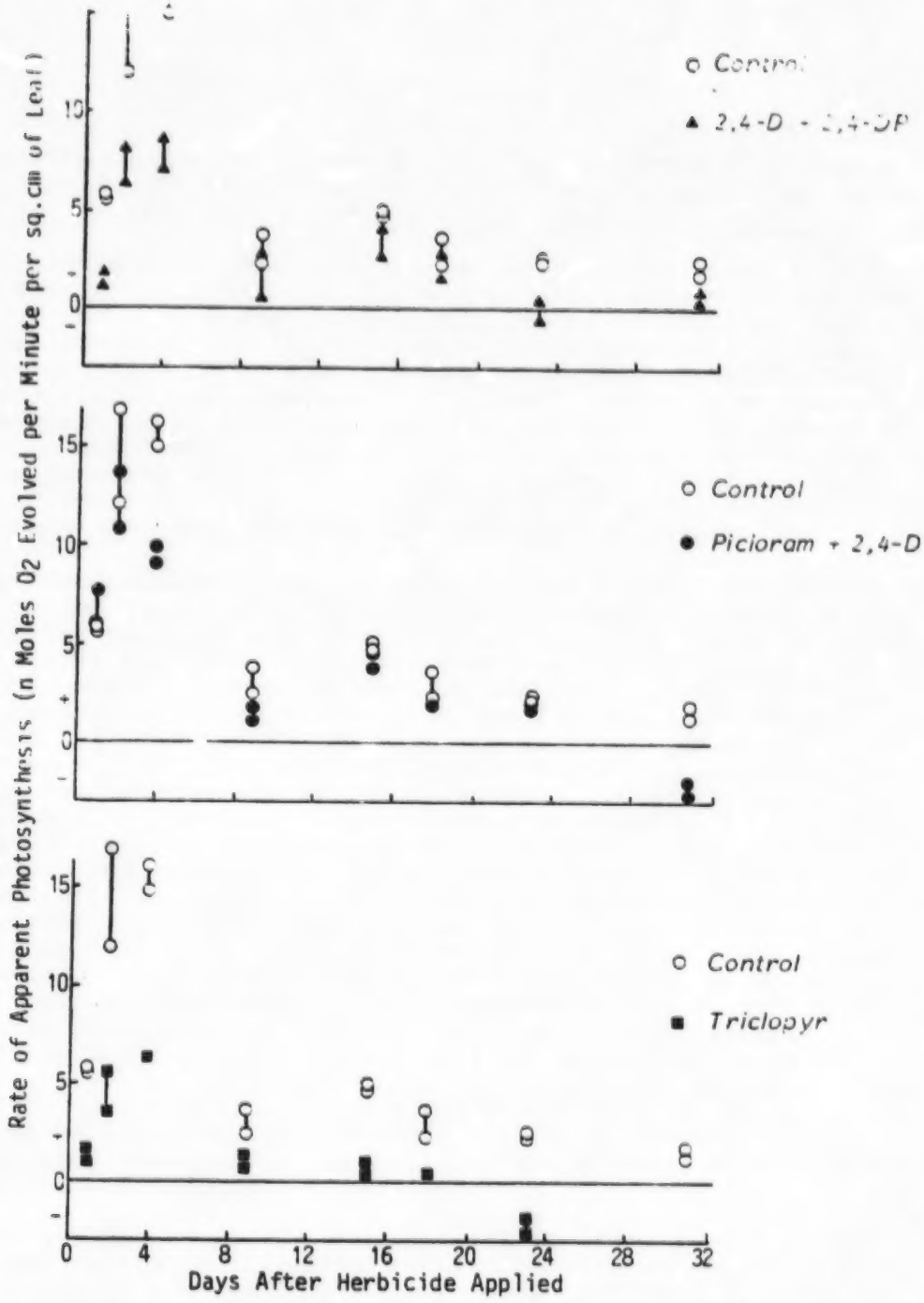


FIGURE 3

EFFECT OF VARIOUS HERBICIDES ON THE APPARENT PHOTOSYNTHESIS OF LEAF DISCS FROM WHITE ASH TREES

Photosynthesis was measured as described in Materials and Methods. A negative rate of oxygen evolution means that oxygen was consumed (i.e. respiration). Two measurements were made for each treatment at various time intervals, and these two values are plotted. At 31 days the leaf material treated with triclopyr disintegrated when touched and no measurements could be taken.

3.4 Effects of the Herbicides 2,4-D plus 2,4-DP; Picloram plus 2,4-D; and Triclopyr on the Respiration of Leaves and Roots from Carolina Poplar and White Ash Trees

Neither of the herbicide combinations (2,4-D plus 2,4-DP; picloram plus 2,4-D; triclopyr alone) had any apparent effect on the respiration of the leaf discs cut from the leaves of Carolina poplar and white ash trees previously sprayed with the chemicals (Figures 4 and 5). Previous reports on the effect of phenoxy-herbicides on plant respiration are confused, with 2,4-D quoted as stimulating leaf respiration of blackjack oak (*Quercus marilandica* Muench.) (Dalrymple and Basler, 1963) and inhibiting oxygen consumption by pea stem segments (Colombo and Ferrari-Bravi, 1982). However, mitochondrial function was reported to be inhibited by both 2,4-D and picloram (Wedding and Black, 1962; Foy and Penner, 1965).

All the herbicide combinations examined had an apparent inhibitory effect on the respiration of isolated root segments from Carolina poplar trees to which the chemicals had been applied as a foliar-spray (Figure 6). This would suggest that the herbicides (one or both in a combination) or phytotoxic herbicide metabolites had been successfully translocated from the leaves to the roots. The fact that leaf respiration did not appear to be inhibited by the herbicides whilst root respiration was, may be explained by the differential sensitivity of plant shoots and roots to phenoxy-herbicides like 2,4-D, with roots being from 100 to 10,000 times more sensitive (for a discussion of this important difference between plant roots and shoots, see Ontario Hydro Research Division Report No 82-153-K). Although proportionately less herbicide is required to kill tree roots than tree foliage, it has been reported that very little herbicide is translocated to the roots after foliar-application. This poor translocation of herbicide from the leaves to the roots is compounded by poor penetration of herbicide into leaf cells after initial application. Davis et al (1968b) found that of the 2,4,5-T and picloram applied to the leaves of live oak (*Quercus virginiana*) 1% and 2% respectively, were absorbed after 48 hours. Of the picloram and 2,4,5-T molecules taken up by the leaves of velvet mesquite (*Prosopis juliflora*), 99% and 97% respectively, remained in the cells of the treated leaves 90 hours after application (Davis et al, 1968a).

Although all the herbicide combinations employed in the present study appeared to inhibit the respiration of root segments from Carolina poplar trees after the herbicides had been applied to the foliage (Figure 6), the mixture of 2,4-D and 2,4-DP appeared to have little or no effect on the respiration of root segments from white ash trees (Figure 7). Although picloram plus 2,4-D and triclopyr caused an inhibition of respiration, the effect was not consistent throughout the course of the whole experiment. It should be noted that the respiration of root segments from Carolina

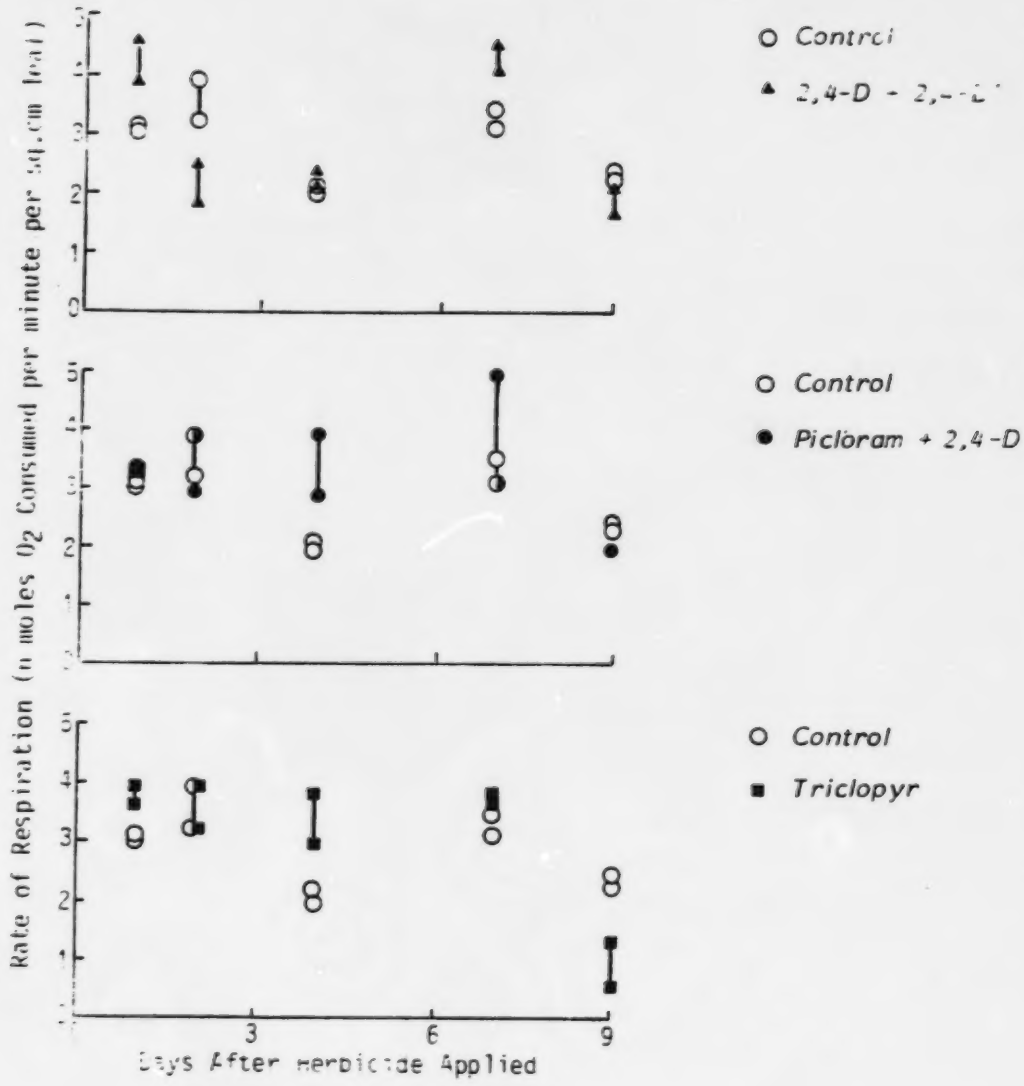


FIGURE 4

EFFECT OF VARIOUS HERBICIDES ON THE RESPIRATION OF LEAF DISCS FROM CAROLINA POPLAR TREES

The respiration of leaf discs was measured as described in Materials and Methods. Two measurements were made for each treatment at each time interval and both values plotted. By 15 days all experimental leaf material was too fragile to be assayed.

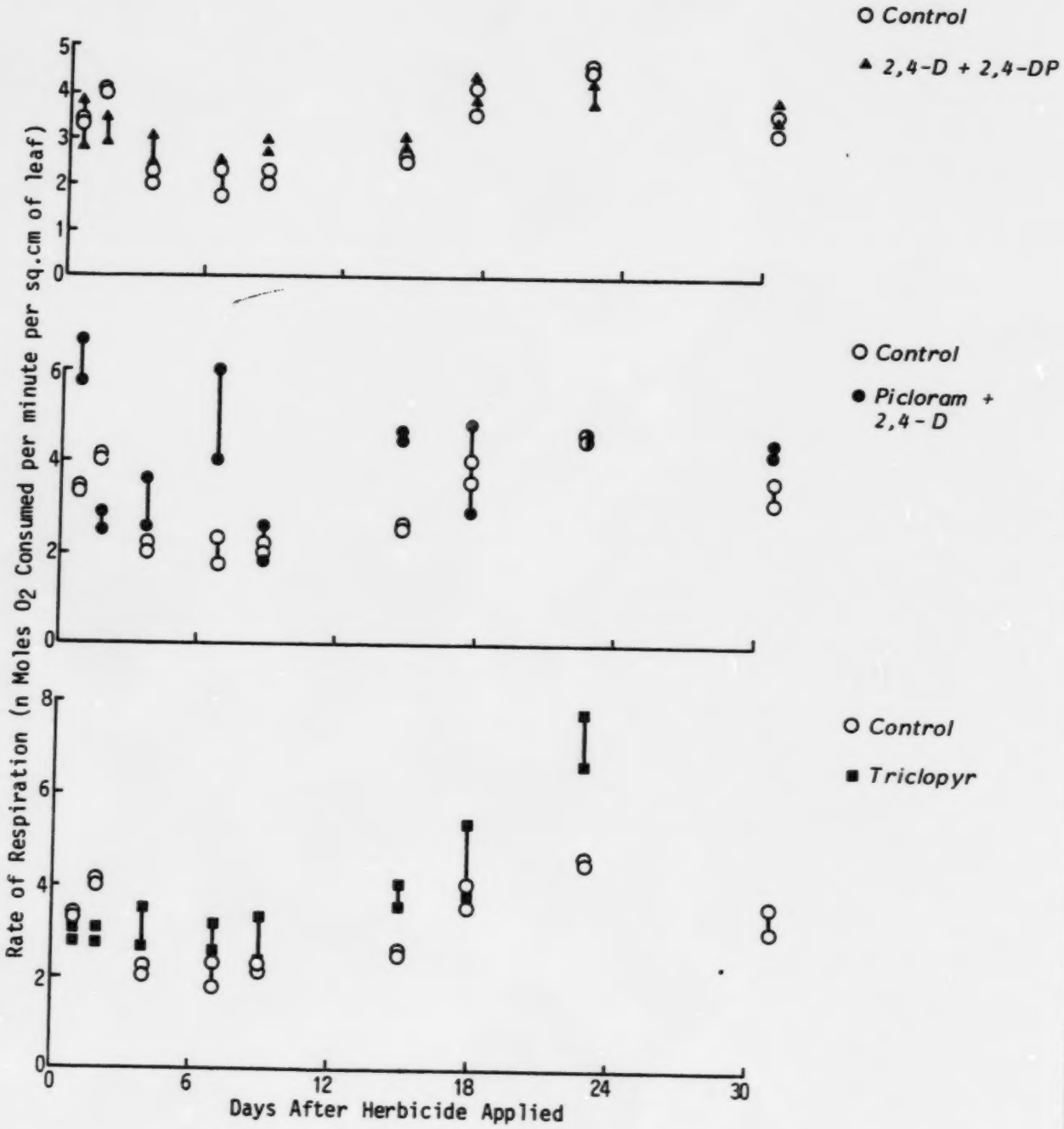


FIGURE 5

EFFECT OF VARIOUS HERBICIDES ON THE RESPIRATION OF LEAF DISCS FROM WHITE ASH TREES

The respiration of leaf discs was measured as described in Materials and Methods. Two measurements were made for each treatment at each time interval and both values plotted. By 31 days the leaves sprayed with triclopyr were too fragile to permit the assay to be undertaken.

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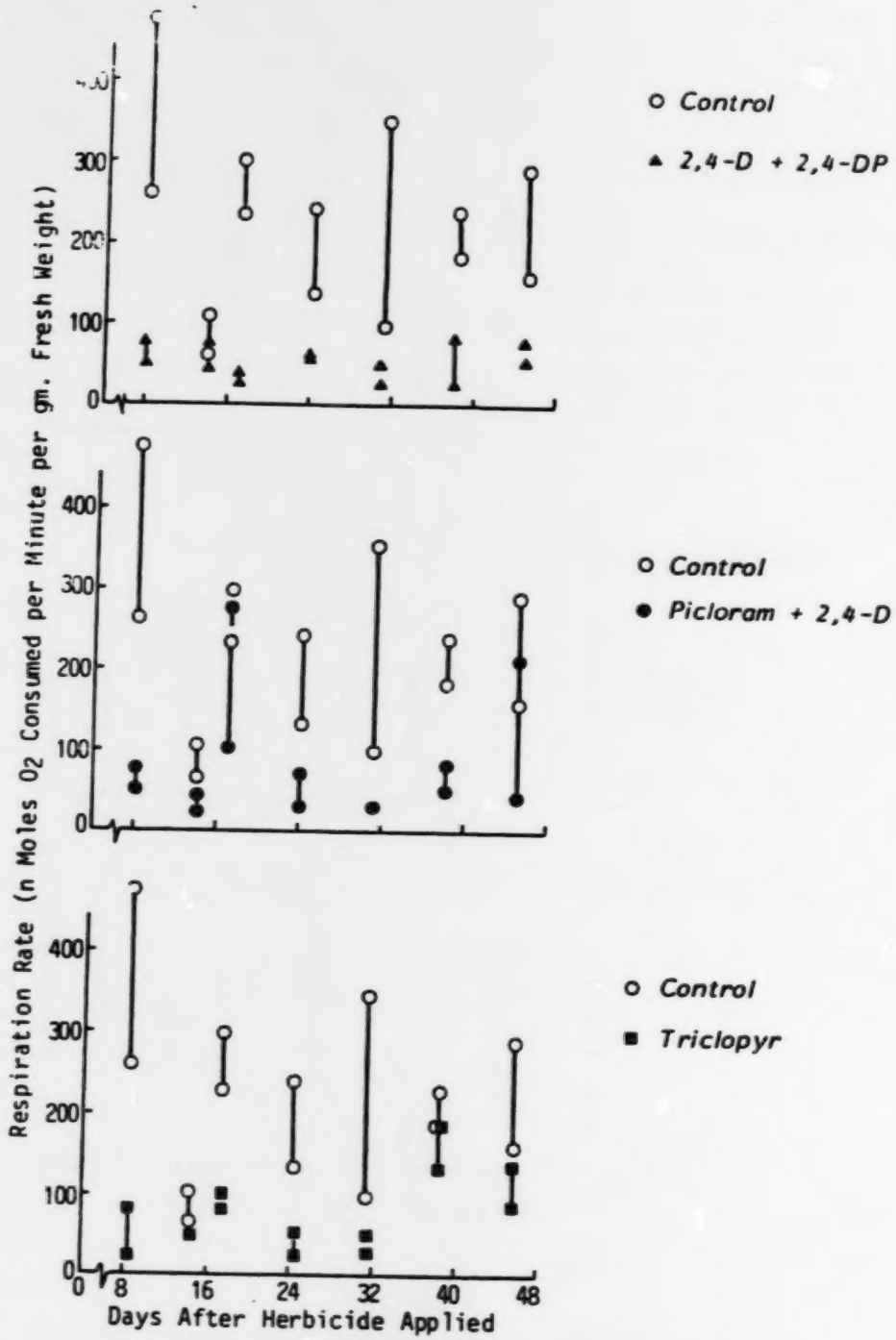

FIGURE 6

EFFECT OF VARIOUS HERBICIDES ON THE RESPIRATION OF ROOT SEGMENTS OF CAROLINA POPLAR TREES

The herbicides were applied to the foliage. The respiration of root segments was measured as described in Materials and Methods, the root segments were surface dried and weighed and the results expressed as a function of fresh weight. Two measurements were made for each treatment at various time intervals, and these values are plotted.

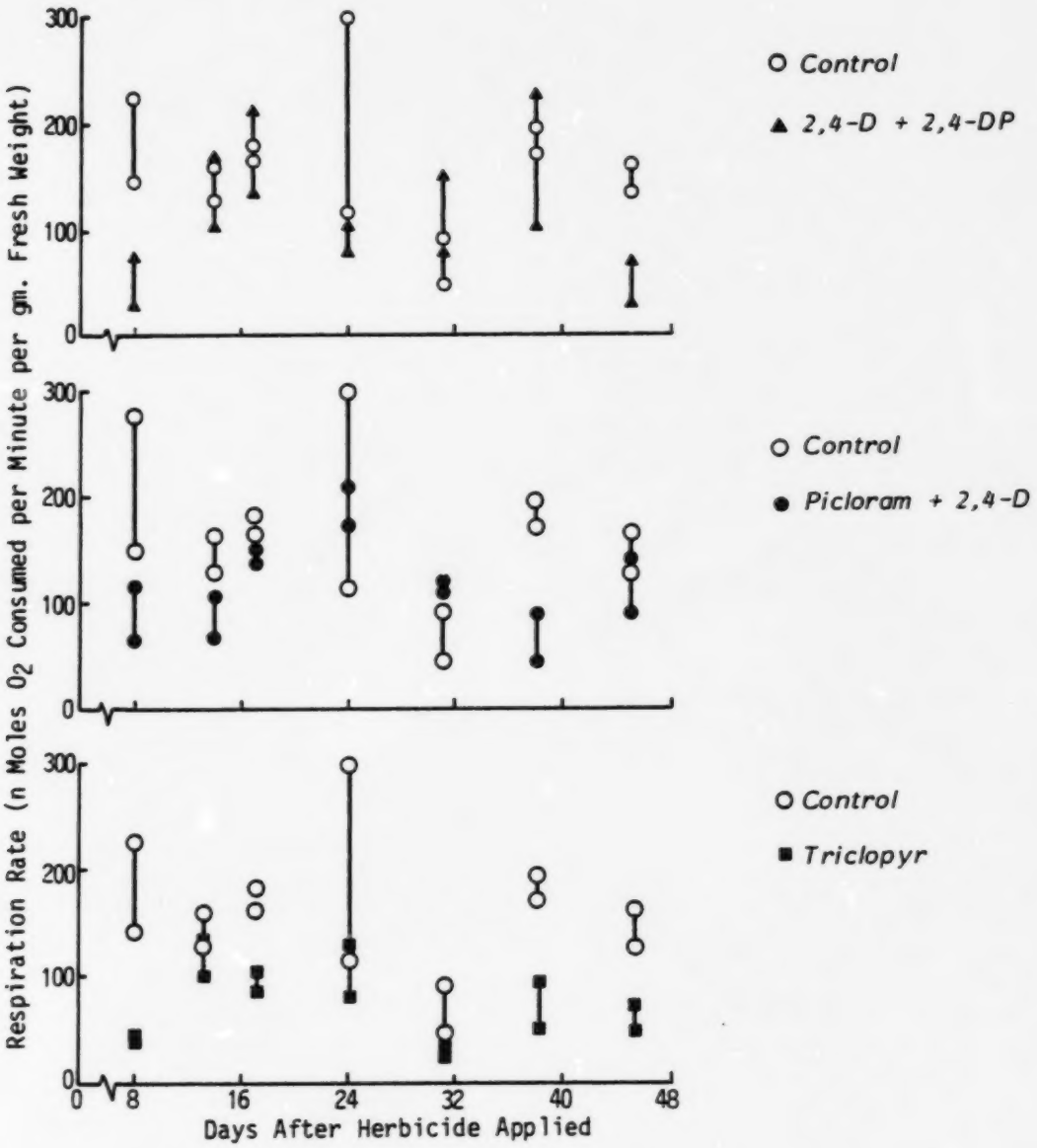

FIGURE 7

EFFECT OF VARIOUS HERBICIDES ON THE RESPIRATION OF ROOT SEGMENTS OF WHITE ASH TREES

The herbicides were applied to the foliage. The respiration of root segments was measured as described in Materials and Methods, the root segments were surface dried and weighed and the results expressed as a function of fresh weight. Two measurements were made for each treatment at various time intervals, and these two values are plotted.

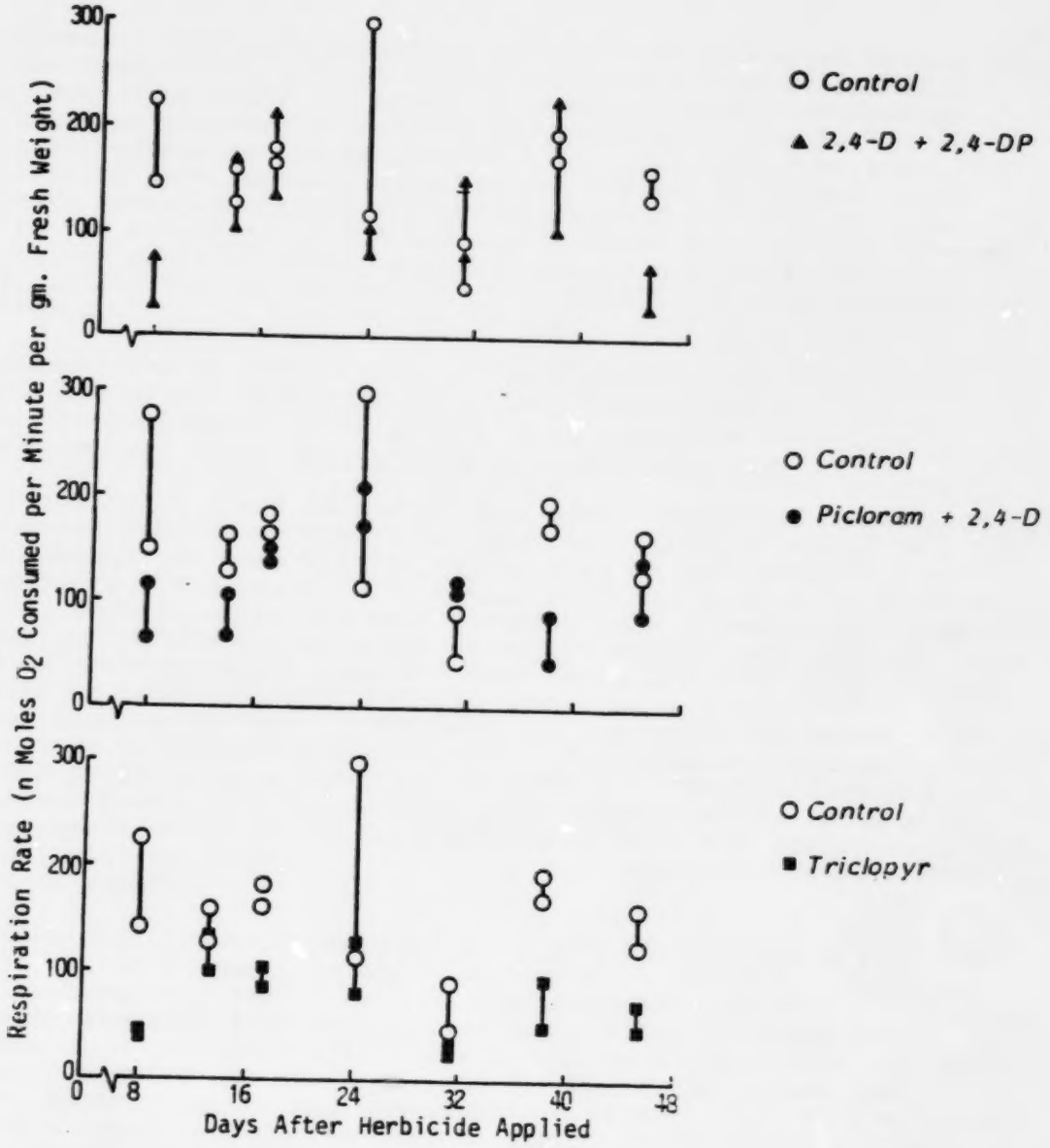

FIGURE 7

EFFECT OF VARIOUS HERBICIDES ON THE RESPIRATION OF ROOT SEGMENTS OF WHITE ASH TREES

The herbicides were applied to the foliage. The respiration of root segments was measured as described in Materials and Methods, the root segments were surface dried and weighed and the results expressed as a function of fresh weight. Two measurements were made for each treatment at various [illegible] and these two values [illegible]

poplar trees, a herbicide-sensitive species, was inhibited by the herbicides used in the present study, whilst the respiration of root segments from white ash trees, a herbicide-resistant species was affected by picloram plus 2,4-D and triclopyr. The lack of any apparent effect by the herbicides 2,4-D plus 2,4-DP on the respiration of root segments of white ash may not be linked to an ineffective translocation of the herbicide from the site of application to the roots, since 2,4-D has been shown to move from treated leaves to the roots of aspen (*Populus tremula* L.) (Eliasson and Hallmen, 1973), but may be due to a low toxicity of this herbicide(s) in the roots.

3.5 A Comparison of the Effects of 2,4-D plus 2,4-DP; Picloram plus 2,4-D; and Triclopyr on Various Aspects of Leaf and Root Metabolism of Carolina poplar (a herbicide-sensitive species) and White Ash (a herbicide-resistant species)

All the results obtained in the present study have been summarized in a somewhat subjective manner in Table 5. Carolina poplar, a herbicide-sensitive tree species, appears to be very sensitive to the mixture of 2,4-D and 2,4-DP, whilst both the mixture of picloram and 2,4-D, and triclopyr are only slightly less effective. Although leaf respiration was not affected by any of the herbicides used, this appears unimportant since leaf and stem death occurred approximately 2 weeks after herbicide application. White ash, a herbicide-resistant species, differs from Carolina poplar in several ways in its response to herbicide application. The mixtures of 2,4-D plus 2,4-DP and picloram plus 2,4-D had no effect on leaf photosynthesis but picloram plus 2,4-D did inhibit root respiration in white ash. Triclopyr appeared to be the most effective herbicide employed on white ash, with an inhibitory effect on both apparent photosynthesis and root respiration.

One obvious difference in the leaf structure of Carolina poplar and white ash is the number of stomata on the upper surface. White ash appears to have very few stomata on the upper leaf surface - indicated by the high stomatal diffusion resistance, whilst Carolina poplar has many stomata on the upper leaf surface. Stomata facilitate the entry of herbicide applied to the leaf into the cells (Robertson and Kirkwood, 1970). Uptake and translocation of herbicide or phytotoxic herbicide metabolite from the leaf to the roots of Carolina poplar appeared to occur with all three herbicide treatments supplied, since root respiration was inhibited. Triclopyr and picloram plus 2,4-D application resulted in inhibition of root respiration in white ash, suggesting that either 2,4-D and 2,4-DP were not translocated to the roots, or they were translocated but had no effect on root respiration. The efficiency of herbicide translocation to the roots of white ash after foliar-application and the sensitivity of the roots to that herbicide in long-term experiments remains to be determined. Foliar-application is a convenient method of supplying herbicide to a woody plant, however, translocation of the herbicide or phytotoxic herbicide metabolite to the roots must be efficient in that destruction of the root system is the primary objective in woody plant control. Inefficient root kill results in

TABLE 5

SUMMARY OF THE EFFECTS OF THE FOLIAR-APPLIED HERBICIDES 2,4-D PLUS 2,4-DP; PICLORAM PLUS 2,4-D; AND TRICLOPYR ON LEAF APPEARANCE AND METABOLISM, AND ROOT RESPIRATION OF CAROLINA POPLAR AND WHITE ASH TREES

The effects of the herbicides 2,4-D plus 2,4-DP; picloram plus 2,4-D; and triclopyr, presented in Tables 1 to 4 and Figures 2 to 7 are summarized in tabular form.

Key: + = small effect; ++ = large effect; ne = no effect

Tree Species	Carolina Poplar			White Ash		
Herbicide Applied	2,4-D+ 2,4-DP	Picloram + 2,4-D	Tri-clopyr	2,4-D+ 2,4-DP	Picloram + 2,4-D	Tri-clopyr
Effect:						
Leaf injury, defoliation	++	++	++	++	+	++
Increase in stomatal diffusion resistance*	++	++	++	+	+	+
Inhibition of apparent photosynthesis	++	+	+	ne	ne	++
Inhibition of leaf respiration	ne	ne	ne	ne	ne	ne
Inhibition of root respiration	++	+	+	ne	+	+

* An increase in leaf stomatal diffusion resistance constitutes an inhibition of transpiration (under certain conditions - for explanation see text).

stem sprouting and root suckering following treatment. Although rapid leaf death (as in Carolina poplar) caused by herbicide application will curtail the translocation of herbicide to the roots, the differing susceptibility of the roots of different woody species to that herbicide may mean that even in a short time enough herbicide reaches the roots to kill them.

4.0 CONCLUSIONS

1. The Carolina poplar trees were sensitive to all three herbicide treatments applied to the foliage (2,4-D plus 2,4-DP; picloram plus 2,4-D; triclopyr) in that young stem and leaf death had occurred within approximately 15 days of herbicide application. In all cases herbicide or phytotoxic herbicide metabolite appeared to have been translocated from the foliage to the roots, since root respiration was inhibited.

2. The white ash trees were more resistant to 2,4-D plus 2,4-DP than Carolina poplar in that stomatal diffusion resistance, apparent photosynthesis and root respiration were apparently not affected (or affected very little) by this mixture. Picloram plus 2,4-D also had no affect (or very little) on stomatal diffusion resistance and apparent photosynthesis, but did inhibit root respiration. Triclopyr inhibited both apparent photosynthesis and root respiration in white ash, and inhibition of the latter would indicate successful translocation of triclopyr or phytotoxic triclopyr metabolite from the leaves to the roots.

3. By 45 days after application of the herbicide treatments to the foliage of Carolina poplar, respiration (although inhibited) still occurred in the roots. Both picloram plus 2,4-D and triclopyr inhibited the respiration of white ash roots, and then only partially. Even after 45 days the white ash roots remained viable. The time course for the (possible) herbicide-induced death of Carolina poplar and white ash roots after foliar-application of herbicide remains to be investigated.

4. Although efficient translocation of herbicide from the leaves to the roots must be accomplished in effective woody plant control, the differing susceptibility of the roots of different woody species to that herbicide may mean that the time preceding herbicide-induced leaf death (after which no more herbicide will be available for translocation) need only be relatively short.

5. From the preliminary results described above, it appears that triclopyr may prove to be more effective in controlling hitherto herbicide-resistant tree species such as white ash, than both herbicide combinations used by Ontario Hydro at present (2,4-D plus 2,4-DP and picloram plus 2,4-D).

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APPENDIX A

HERBICIDE COMPOSITION

2,4-D Plus 2,4-DP

The mixture of 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4-DP (2,4-diclorophenoxypropionic acid) was obtained from Union Carbide Agricultural Products, batch number S77107 under the trade name Desormone 700. The herbicide mixture contained the butoxyethanol ester derivatives of 2,4-D (350 g/L acid equivalent) and 2,4-DP (350 g/L acid equivalent). The stock solution of herbicide was diluted 100 times with tap water and sprayed on the tree foliage as described in Materials and Methods.

Picloram Plus 2,4-D

The mixture of picloram (4-amino 3,5,6-trichloropicolinic acid, triisopropanolamine salt) and 2,4-D (2,4-dichlorophenoxyacetic acid, triisopropanolamine salt) under the trade name Tordon 101 was obtained from Dow Chemical of Canada Limited via various intermediaries (lot number unknown). The stock solution contained 60 g/L acid equivalent of picloram and 240 g/L acid equivalent of 2,4-D. This stock solution was diluted 100 times with tap water before application to the tree foliage.

Triclopyr

The stock solution of triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid was obtained from Dow Chemical of Canada Limited under the trade name Garlon 4 (or Dowco 233) (lot number unknown). This herbicide is registered for use in the USA but not in Canada (other than for research purposes). Garlon 4 is an oil soluble, water emulsifiable ethylene glycol butyl ether ester formulation. The stock solution of Garlon 4 contained 480 g/L acid equivalent of triclopyr. This stock solution was diluted 133 times with tap water prior to application to the tree foliage.

APPENDIX B

EFFECT OF THE FOLIAR-APPLIED HERBICIDES 2,4-D PLUS 2,4-DP; PICLORAM PLUS 2,4-D; AND TRICLOPYR ON THE STOMATAL DIFFUSION RESISTANCE (r_s) OF THE UPPER AND LOWER LEAF SURFACES OF CAROLINA POPLAR

Stomatal diffusion resistance of the upper and lower surface of the same leaf was measured on three leaves chosen at random as described in Materials and Methods. Total (whole) leaf stomatal diffusion resistance was calculated using the formula (from Slavik, 1974):

$$\frac{1}{r_{s(total)}} = \frac{1}{r_{s(upper)}} + \frac{1}{r_{s(lower)}}$$

In moving air there is a strong (inverse) relationship between stomatal diffusion resistance and transpiration (see Figure 1). For the three samples taken, the mean values are quoted ± one standard deviation.

Herbicide Applied	Surface	Sample	Days after herbicide applied to leaves				
			1	2	4	7	9
			r_s (sec cm^{-1})				
Control (no herbicide applied)	Upper	1	16.6	23.2	88.9	12.3	21.8
		2	12.0	19.5	40.9	8.7	7.3
		3	9.5	10.9	23.7	6.5	6.3
		$\bar{X}$ ± sd	12.7 ± 3.6	10.9 ± 8.6	51.2 ± 33.8	9.2 ± 2.9	11.8 ± 8.7
	Lower	1	11.8	6.2	6.5	4.4	4.3
		2	9.5	5.5	10.3	3.3	3.2
		3	9.5	4.3	6.6	3.7	2.4
		$\bar{X}$ ± sd	10.3 ± 1.3	5.3 ± 0.9	7.8 ± 2.1	3.8 ± 0.6	3.3 ± 0.9
	Upper & Lower	1	6.9	4.9	6.1	3.2	3.6
		2	5.3	4.3	8.2	2.4	2.2
		3	4.8	3.1	5.2	2.3	1.7
		$\bar{X}$ ± sd	5.6 ± 1.1	4.1 ± 0.9	6.5 ± 1.6	2.6 ± 0.5	2.5 ± 0.9
2,4-D + 2,4-DP	Upper	1	75.8	89.6	121.7	56.7	156.1
		2	75.1	59.3	102.5	26.9	133.9
		3	68.7	43.8	77.0	21.7	121.1
		$\bar{X}$ ± sd	73.2 ± 3.9	64.2 ± 23.3	100.4 ± 22.4	35.1 ± 18.9	137.0 ± 17.7
	Lower	1	97.8	60.1	64.2	47.2	158.1
		2	93.9	27.9	106.8	74.8	141.1
		3	68.6	22.4	25.5	48.1	137.0
		$\bar{X}$ ± sd	86.8 ± 15.9	36.8 ± 20.3	65.2 ± 41.2	56.6 ± 15.7	145.4 ± 11.2
	Upper & Lower	1	43.5	36.0	42.0	25.7	78.5
		2	41.7	18.9	52.3	19.8	68.7
		3	34.9	14.8	18.6	14.9	64.3
		$\bar{X}$ ± sd	40.0 ± 4.5	23.3 ± 11.2	37.6 ± 17.3	20.2 ± 5.4	70.5 ± 7.3
Picloram + 2,4-D	Upper	1	85.6	97.7	90.3	85.2	325.4
		2	78.5	72.5	73.3	82.6	303.0
		3	55.1	49.0	53.6	76.7	298.7
		$\bar{X}$ ± sd	56.4 ± 44.6	73.1 ± 24.3	72.4 ± 18.4	81.5 ± 4.4	309.1 ± 14.3
	Lower	1	77.6	65.7	22.3	79.7	754.7
		2	104.8	17.1	21.2	72.5	735.9
		3	53.9	12.5	5.1	83.7	686.6
		$\bar{X}$ ± sd	78.8 ± 25.5	31.8 ± 29.5	16.2 ± 9.6	78.6 ± 5.7	725.7 ± 35.2
	Upper & Lower	1	40.0	39.3	17.9	41.2	227.5
		2	45.5	13.8	16.4	38.6	214.7
		3	27.4	9.9	4.6	40.0	208.1
		$\bar{X}$ ± sd	37.6 ± 9.3	21.0 ± 15.9	12.9 ± 7.3	39.9 ± 1.3	216.7 ± 9.9
Triclopyr	Upper	1	74.5	62.6	134.5	88.1	110.9
		2	49.7	52.9	116.3	63.2	97.3
		3	44.7	27.4	96.5	40.6	29.9
		$\bar{X}$ ± sd	56.3 ± 15.9	47.6 ± 18.3	115.8 ± 19.0	63.9 ± 23.8	79.4 ± 43.4
	Lower	1	53.9	83.5	95.2	68.3	66.8
		2	51.1	45.6	47.2	64.1	64.2
		3	46.1	65.3	36.1	35.6	57.2
		$\bar{X}$ ± sd	50.2 ± 3.7	64.8 ± 18.9	59.5 ± 31.4	55.9 ± 17.8	62.8 ± 4.9
	Upper & Lower	1	30.8	35.8	55.7	38.5	41.7
		2	24.9	24.5	33.6	32.9	38.7
		3	22.5	19.2	26.7	18.9	19.6
		$\bar{X}$ ± sd	26.1 ± 4.3	26.5 ± 8.5	38.5 ± 15.3	30.1 ± 10.1	33.3 ± 11.9

APPENDIX C

EFFECT OF THE FOLIAR-APPLIED HERBICIDES 2,4-D PLUS 2,4-DP; PICLORAM PLUS 2,4-D; AND TRICLOPYR ON THE STOMATAL DIFFUSION RESISTANCE (r_s) OF THE UPPER AND LOWER LEAF SURFACES OF WHITE ASH TREES

Same conditions as described in the legend to Appendix B. Where no values are quoted, measurements could not be taken because of the poor physical condition of the leaves. For the three samples taken, the mean values are quoted ± one standard deviation.

Herbicide Applied	Leaf Surface	Sample	Days after herbicide applied: 1	2	4	7	9	11	15	25	30
Control (no herbicide applied)	Upper	1	256.9	395.2	296.0	131.4	302.5	193.9	161.2	116.0	93.1
		2	220.3	341.8	291.3	110.5	247.2	137.0	102.9	85.4	82.5
		3	269.4	319.3	193.2	108.7	225.2	107.9	90.6	77.8	64.9
		$\bar{X}$±sd	248.9±25.5	352.1±38.9	260.2±58.0	116.9±12.6	258.3±39.8	146.3±43.8	118.3±37.7	93.1±20.3	80.2±14.2
	Lower	1	18.9	22.7	11.9	17.7	27.9	34.3	2.6	11.9	20.9
		2	16.7	9.2	9.1	9.4	20.1	21.2	2.4	6.7	17.3
		3	7.3	3.6	4.3	3.8	8.5	18.8	1.6	6.4	7.3
		$\bar{X}$±sd	14.3± 6.1	11.8± 9.8	8.4± 3.9	10.3± 6.9	16.8± 7.3	24.7± 8.4	2.2± 0.5	8.3± 3.1	15.2± 7.1
	Upper & Lower	1	17.6	21.4	11.5	15.6	20.5	29.1	2.6	10.8	17.1
		2	15.3	8.9	8.8	8.7	18.6	18.3	2.3	6.2	14.3
		3	7.1	3.5	4.2	3.7	8.2	15.9	1.6	5.9	6.5
		$\bar{X}$±sd	13.4± 5.5	11.3± 9.2	8.2± 3.7	9.3± 5.9	15.7± 6.6	21.1± 7.0	2.2± 0.5	7.6± 2.8	12.7± 5.5
2,4-D Plus 2,4-DP	Upper	1	141.4	346.6	221.8	211.3	550.5	438.7	170.7	99.1	
		2	124.8	314.7	172.8	109.5	277.3	425.9	138.6	71.3	
		3	102.0	270.5	137.8	69.2	275.3	252.4	89.8	61.8	
		$\bar{X}$±sd	122.7±19.8	310.6±38.3	177.5±42.2	130.0±73.2	367.8±19.2	372.3±104.1	133.0±40.8	77.4±19.4	
	Lower	1	36.9	44.4	27.7	49.9	32.8	87.9	43.0	60.7	
		2	29.8	37.3	24.0	46.6	70.8	77.0	39.7	33.4	
		3	14.7	34.5	27.5	35.8	25.9	24.6	18.2	27.1	
		$\bar{X}$±sd	27.1±11.4	38.7± 5.1	26.4± 2.1	44.1± 7.4	43.5±24.8	63.3±33.9	33.6±13.5	40.4±17.8	
	Upper & Lower	1	29.3	39.4	24.6	40.4	30.9	73.3	34.4	37.6	
		2	24.0	33.4	21.1	32.7	56.4	65.2	30.8	22.8	
		3	12.8	30.6	22.9	23.6	43.7	24.4	15.1	18.8	
		$\bar{X}$±sd	22.1± 8.4	34.4± 4.6	22.9± 1.8	32.2± 8.4	37.0±17.2	53.6±27.3	26.8±10.2	26.4± 9.9	
Picloram + 2,4-D	Upper	1	144.9	355.9	279.2	288.0	173.3	285.4	133.2	99.1	114.7
		2	140.8	326.8	185.7	174.4	137.1	158.3	94.3	87.6	106.7
		3	134.0	309.8	168.9	155.6	131.0	178.9	87.6	76.8	68.6
		$\bar{X}$±sd	139.9± 5.5	330.9±23.3	211.3±59.5	206.0±71.6	147.1±22.8	207.5±68.2	105.0±24.6	87.8±11.1	96.7±24.7
	Lower	1	10.3	29.6	33.3	39.6	42.4	42.1	21.3	19.2	49.1
		2	10.2	12.9	21.2	33.9	23.4	31.8	10.4	15.9	37.9
		3	8.3	7.4	51.5	27.6	20.8	20.6	9.1	9.1	35.9
		$\bar{X}$±sd	9.6± 1.1	16.7±11.6	29.4±10.2	33.7± 5.9	28.9±11.7	31.5±10.8	13.6± 6.7	14.8± 5.2	40.9± 7.1
	Upper & Lower	1	9.6	27.3	29.7	34.8	34.0	36.7	18.4	16.1	34.4
		2	9.5	12.5	19.0	28.4	20.0	26.5	9.4	13.5	27.9
		3	7.8	7.2	39.4	23.5	17.9	18.4	8.3	8.2	23.6
		$\bar{X}$±sd	8.9± 1.0	15.7±10.4	29.4±10.2	28.9± 5.7	24.0± 8.7	27.2± 9.2	11.9± 5.5	12.6± 4.0	28.7± 5.4
Triclopyr	Upper	1	202.8	275.9	243.2	269.2	366.2	290.2			
		2	181.6	312.5	224.9	229.7	268.7	308.0			
		3	111.8	187.0	205.6	184.0	250.2	248.5			
		$\bar{X}$±sd	165.4±47.6	258.5±64.5	224.6±18.8	227.7±42.6	295.0±62.3	282.2±30.5			
	Lower	1	17.7	76.0	46.5	63.2	74.8	113.9			
		2	12.1	27.7	23.2	88.0	35.2	73.9			
		3	6.2	27.2	13.6	40.5	21.3	54.5			
		$\bar{X}$±sd	12.0± 5.8	43.6±28.0	27.8±16.9	63.9±23.8	43.8±27.7	80.8±30.3			
	Upper & Lower	1	16.3	59.6	39.1	51.2	62.1	81.8			
		2	11.4	25.4	21.1	63.7	31.2	59.6			
		3	5.9	23.8	12.7	33.2	19.7	44.7			
		$\bar{X}$±sd	11.2± 5.2	36.3±20.2	24.3±13.5	49.4±15.3	37.7±21.9	62.0±18.7			

POOR

QUALITY

ORIGINAL